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Publication date:
2015

QUANTITATIVE PHASE IN MICROSCOPY: BACK-TO-BASICS MEASUREMENTS

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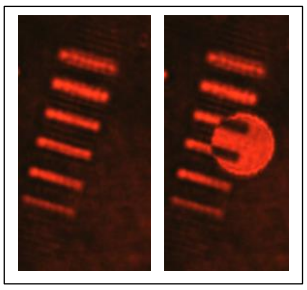
KEYWORDS: Quantitative phase imaging, spatial light modulators, Generalized phase contrast

1. BASIC VERSUS DERIVED MEASUREMENT

Phase measurement is an attractive label-free technique offering quantitative information with minimal disturbance and simpler sample preparation, which is free from uncertainties due to photobleaching and biological absorption variabilities attending most tagging techniques. A basic measurement determines the amount of a physical quantity by counting how many standard units reproduces the quantity being measured (e.g., a basic length measurement uses a calibrated ruler to count how many meters reproduces a given length). On the other hand, a more elaborate length measurement can e.g., use the time-of-flight of reflected ultrasonic pulses to calculate the length using the speed of sound. Most measurements in quantitative phase microscopy are, arguably, of the elaborate type that calculate the phase based on the properties of light propagation. This work introduces a back-to-basics approach in phase measurements (phase measurements in microscopy do not measure absolute phase but the phase difference between different parts of an observation field). A basic phase measurement could count how many calibrated phase units are needed to reproduce an unknown phase or, alternately, count how many such calibrated phase units are needed to cancel the observed phase difference. We previously used spatial light modulators (SLMs) for adaptive phase imaging[3]. Here we use SLMs as ”phase rulers” to measure phase differences by determining how much SLM phase cancels a given phase difference.

2. MEASUREMENT AND RESULTS



Determining the SLM phase that cancels a given phase difference was done by placing the SLM and sample planes in conjugate planes. We built an optical system after the camera port of a brightfield microscope to project the sample plane onto the SLM plane and then used a phase visualization system to verify phase cancellation. The figure shows the phase visualization output to illustrate the measurement process: left- without adding SLM phase; right: with superimposed circular SLM phase adjusted to cancel the initial phase (the unknown phase is determined based on SLM calibration).

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